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PRINCIPAL INVESTIGATOR: Nicanor Obaldia, DVM, M.S.

CONTRACTING ORGANIZATION: Promed Trading, SA
Miami, Florida 33126

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Nicanor Obaldia, DVM, M.S.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Promed Trading, SA
Miami, Florida 33126

E-MAIL:

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A C2A clone of a Mefloquine resistant *Plasmodium falciparum* strain was succesfully adapted to splenectomized and intact *Aotus*. Chloroquine resistance reversal was achieved in 3/3 *Aotus* infected with the AMRU-1 strain of *Plasmodium vivax* by using chloroquine at 10mg/kg and prochlorperazine at 20 mg/kg in combination. Artelinic Acid (WR255663AK;BM04131) when given orally at 2 mg/kg x three days suppressed infections of the AMRU-1 (CQR) but cleared SAL-1 strains of *P. vivax* in Aotus monkeys. Artelinic Acid (WR255663AK;BM 04131) administered orally at 2-24 mg/kg x three days was effective against infections of *P. falciparum* FVO strain in Aotus monkeys. Orally or intravenously administered falcipain (APC3317) was ineffective against infections of *P. falciparum FVO*. Artelinic Acid and Artesunate were effective against infections with *P. falciparum* FVO in Aotus monkeys. Passive transfer of anti-EBA-175 Region II protein monoclonal antibodies was not effective at controlling parasitemia in *Aotus* monkeys infected with *P. falciparum.* Immunization with a plasmid encoding region II of EBA-175 followed with by a EBA-175 recombinant protein boost partially protected Aotus monkeys against *P. falciparum* malaria. Topical ocular administration of a plasmid DNA vaccine encoding an AMA-1 *P. falciparum* blood stage antigen did not induce an immune response in Aotus monkeys.

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INTRODUCTION

Each year there are 300-500 million new infections and 2-5 million deaths attributable to malaria that occur primarily in countries in the tropics, particularly in sub-Saharan Africa **(4)**. During the past 10-20 years the malaria problem has intensified in some parts of the world because parasites have developed resistance to drugs used for treatment and prevention; the anopheles mosquito, which transmits the parasite to humans, has developed resistance to insecticides, and control efforts have been reduced as resources have diminished in some developing countries **(5)**.

The use of *Aotus lemurinus lemurinus* (Panamanian *Aotus* monkey), cariotypes VIII and IX (11) as a model to study malaria drug resistance and vaccine efficacy, have been ongoing at Gorgas Memorial Laboratory since 1976, due in part to the availability of this monkey in Panama **(15)**, and also to the increasing drug resistance exhibited by the highly pathogenic *Plasmodium falciparum* parasites in Asia, Africa, and Latin America, and more recently *Plasmodium vivax* in the Melanesian and Indonesian archipielago **(16)**. Previously, Schmidt **(21, 22)** used the Colombian *Aotus* as the experimental host for antimalarial drug studies, but embargoes imposed by South American countries on the exportation of monkeys in the mid 1970's seriously restricted the use of *Aotus* for biomedical research in the United States, and in 1976 the project was transferred to Gorgas Memorial Laboratory where Panamanian *Aotus* were available for research. Since then, three strains of *P. falciparum*, Vietnam Smith, Uganda Palo Alto, and Vietnam Oak Knoll, had been adapted to Panamanian *Aotus*. These strains exhibit diverse susceptibility and/or resistance to standard antimalarial agents.

The course of untreated infections in Panamanian *Aotus* has been characterized and compared with that in *Aotus* of Colombia **(20)**. Overall, the virulence of these strains was less in Panamanian than in Colombian owl monkeys, as indicated by lower mortality rates of Panamanian monkeys during the first 30 days of patency. Maximum parasitemias of the Vietnam Smith and Uganda Palo Alto strains were, however, significantly higher during the first 15 days of patency in Panamanian than in Colombian owl monkeys. These quantitative differences in infection parameters between Panamanian and Colombian owl monkeys have not invalidated the use of the former for evaluation of new antimalarial drugs.

Numerous candidate antimalarial drugs of diverse chemical classes have been evaluated against trophozoite-induced infections of one or more *P. falciparum* strains during the course of these contracts. In seeking alternatives to primaquine, two 8-aminoquinolines proved to be active against the blood stages of *P. falciparum* **(2, 13)**. Desferrioxamine, an iron-

specific- chelating agent, was shown to suppress parasitemias of the virulent Uganda Palo Alto strain of *P. falciparum* **(18)**. The *in vitro* activity of two halogenated histidine analogs was not confirmed by evaluation against *P. falciparum* infections in owl monkeys **(17)**.

Chloroquine-resistance of *P. falciparum* represents the greatest challenge in developing effective antimalarial drugs. Reversal of chloroquine-resistance in *P. falciparum, in vitro*, was achieved by the co-administration of verapamil (a calcium channel blocker) plus chloroquine **(12)**. Other in vitro studies have shown that there is a significantly greater efflux of chloroquine from erythrocytes containing falciparum parasites resistant to chloroquine than from red cells parasitized by chloroquine-sensitive falciparum malaria **(9)**. Calcium channel blockers appear to prevent this active efflux of chloroquine, thus allowing the drug to accumulate to parasiticidal levels.

Based upon the success of *in vitro* reversal of chloroquine-resistance, trials were initiated to determine if resistance could be reversed in *Aotus* infected with the chloroquine-resistant Vietnam Smith strain of *P. falciparum*. Six calcium channel blockers, or similarly acting drugs, were co-administered with chloroquine in diverse regimens. The desideratum of chloroquine-resistance reversal was administration of a single course of treatment, with parasite clearance and infection cure. Suppression of parasitemia was obtained during an initial course of treatment, but parasite clearance and cure occurred in some instances only after re-treatment. Such infection parameters were similar to those in monkeys with self-limited infections and cure could be attributed to acquired immunity.

Limited trials with desipramine, Norpramin , a tricyclic psychotropic drug, demonstrated the feasibility of reversing chloroquine-resistance in vivo **(1)**. parasite clearance was obtained, but the infection was not cured.

Subsequently, in vivo reversal of chloroquine resistance was obtained with combinations of chloroquine plus chlorpromazine or prochlorperazine. Such reversal was exhibited by rapid suppression and clearance of parasitemia, resulting in infection cure without retreatment **(10)**.

Evaluation of two oil-soluble derivatives of artemisinin, artemether and arteether, demonstrates that both possess similar activity to cure infections of a multi-drug resistant *P. falciparum* strain in *Aotus* **(23)**.

Some strains of *P. vivax* from Melanesia and the Indonesian archipelago have demonstrated resistance to treatment with chloroquine **(14, 19)**. Unlike chloroquine-resistant falciparum malaria, there exists no easy alternative to chloroquine-resistant strains of vivax malaria. Using WR 238605 alone or in combination with chloroquine in Panamanian *Aotus* monkeys it was demonstrated that WR238605 is a an alternative treatment for chloroquine-resistant vivax malaria **(16)**. The compound WR 238605 is a

primaquine analog developed by the US Army as a better tolerated, more effective replacement for primaquine.

Both the purpose and methods of approach of the present work remains essentially unchanged since 1976, viz to ascertain the antimalarial activity of drugs against *P. falciparum* and *P. vivax* in *Aotus*. The method of approach may vary on an ad hoc basis, such as administering a combination of drugs.

The long term goal of the second part of this project is to develop fully protective plasmid DNA vaccines that induce protective immune responses against the sporozoite, liver and erythrocytic stages of *P. falciparum*. If successful, it will establish, for the first time, that plasmid DNA vaccines can protect non-human primates, a critical step forward for the use of plasmid DNA vaccines in humans.

Vaccines are aimed at inducing immune responses that disrupt the complex cycle of the parasite at one more points: anti-sporozoite antibodies that prevent invasion of hepatocytes; cytotoxic T lymphocytes, cytokines, and antibodies that eliminate infected hepatocytes; antimerozoite antibodies that prevent invasion of erythrocytes; antibodies that neutralize parasite exoantigens known to induce harmful cytokine responses; antibodies that attack infected erythrocytes; cytokines that kill parasites within erythrocytes; and, anti-sexual stage antibodies that prevent the development of sporozoites in the mosquito.

Previous trials of malaria blood stage vaccines have shown that the Panamanian *Aotus*\|*P. falciparum* model to be suitable for this purpose. **(6-8)**.

Immunogenicity studies of a plasmid DNA vaccines encoding the circumsporozite *P. yoelli* rodent malaria gene (PyCSP) in Panamanian *Aotus* monkeys demonstrated that the intradermal route of inoculation (ID) induces a higher level of antibodies than the intramuscular route (IM). Antibody levels induced in this manner reached a peak at week 9 and titers declined to 50% their peak value by week 14. When boosted at week 46 antibody levels increase 4 fold by week 49. This was comparable to antibodies generated with a Multiple Antigen synthetic peptide vaccine (MAP) delivered with an adjuvant **(4)**

We have used this inmunization scheduled to test single or multi-gene DNA plasmid vaccines in *Aotus* monkeys. Additionally we have tested the ability of recombinant cytokines to enhance the immunogenicity and protective efficacy of the DNA vaccines. Preliminary studies (previously described in the 1996 Annual Report) using a small group of *Aotus l. lemurinus* (n = 3) demonstrated partial, but incomplete, protection with a DNA vaccines for either AMA-1 or EBA-175 alone. These studies indicated that animals which received the vaccine candidates, had a short, but

apparent significant delay in the onset of parasitemia {approximately 33% (1 of 3) self-cured, whereas none of the control animals did}. However, since the number of animals per group in each of these pilot studies were small, it was not possible to determined the absolute efficacy of these candidate vaccines, but these experiments suggested to the investigators that further studies were warranted. MSP-1, when used as a protein/peptide vaccine formulation, provided protection from a *P. faciparum* infection in *Aotus* monkeys and we have demonstrated that, in mice and in Rhesus monkeys, the cytokine GM-CSF augmented both immunogenicity of a malaria DNA vaccine (personal communication. W. Weiss). We have now completed a pilot experiment to determine if *Aotus* Granulocyte-Macrofage-Colony Stimulating Factor (aGM-CSF) can augment immunogenicity and protective efficacy of a multi-gene erythrocytic vaccine.

We have also tested the effect of prior *P. falciparum* infection on the immunogenicity of a DNA vaccine, obtaining partial protection in 67% of the monkeys.

The purpose of this report is to: 1) Present data on the evaluation of potential antimalarial activity of drugs in the pre-clinical model of *Aotus l. lemurinus* (Panamanian night monkey) experimentally infected with *P. falciparum* or *P. vivax*, and 2) data on plasmid DNA malaria vaccine experiments. These studies were supported by the U.S. Army and the U.S. Navy Malaria Programs.

BODY

I. Experimental Methods

The first aim of this project is to evaluate the potential antimalarial activity of drugs, or combination thereof, in the preclinical model of *Aotus* experimentally infected with *P. falciparum* (or *P. vivax*). Specifically, the vertebrate host is *A. l. lemurinus*, the Panamanian night monkey. These animals are either feral, laboratory adapted or laboratory born. No naturally acquired, human plasmodium infection has been reported in *Aotus*. The Vietnam Smith/RE strain of *P. falciparum* was adapted to *Aotus* of Colombian origin in 1971 **(21)** and in Panamanian *Aotus* in 1976. **(20)**. The course of untreated infections, essential for comparison with treated infections, has been documented in Panamanian *Aotus* **(20)**. This plasmodium strain is resistant to maximally tolerated doses of chloroquine, pyrimethamine, and quinine **(22)**.

To initiate an experiment, infected blood (with 2.5% sodium citrate as the anticoagulant) from an untreated *Aotus* was diluted appropriately in chilled saline (0.85%), such that each milliliter contained 5,000,000 parasites. This amount was inoculated into the saphenous vein of experimental and control monkeys.

Blood films, prepared and examined daily beginning on the first post-inoculation day, were stained with Giemsa. Parasitemias were evaluated as follows: negative, if no parasites were detected on a thick blood film after examination for at least 5 minutes; <10 parasites per cmm, if positive only on the thick blood film; parasite enumeration was by the Earle-Perez method and reported as the number of parasites per cmm. **(3)**

Blood films from untreated *Aotus*, serving as passage and/or control subjects, were prepared and examined daily during the primary patent period, and daily thereafter for at least three consecutive days after parasites could last be detected on thick blood films. When parasitemia had cleared, films were made and examined twice weekly until a total of 100 negative days had been recorded. If recrudescence occurred, blood films were obtained again on a daily basis.

Parasitemias were evaluated daily during the treatment period and until blood films were negative for at least seven consecutive days. The frequency of smearing was then reduced to two times per week (Monday and Thursdays or Tuesdays and Fridays). If no recrudescences occurred during a 100 day examination period, the infection was considered to have been cured.

Drug doses were calculated as mg base per kg of body weight. Stock solutions of water soluble compounds, at appropriate concentrations, were

prepared with distilled water and stored at 8° C for the treatment period. If a compound was water insoluble, a suspension of the requisite amount of drug was prepared daily with 0.3% methylcellulose (in distilled water).

Oral administration of drugs was by gastric intubation with a 14 French catheter. The total volume of fluid administered, drug solution or suspension, and rinse was 14 ml.

Response to treatment was categorized as clearance and cure, clearance and recrudescence, or suppression withouth clearance. The day of clearance was defined as the first of three consecutive days in which the thick blood films were parasite negative. The day of recrudescence was the first of three consecutive days of positive thick blood films after a period of clearance. Suppression was defined as a transient decrease in the parasite count post-treatment without clearance.

The second objective of this project is to evaluate plasmid DNA vaccines against the blood and sporozoite stages of *P. falciparum* and against the blood stages of *P. vivax* in the Panamanian *Aotus* model. To this end we have evaluated single and multigene DNA vaccines of *both P. falciparum* and *P. vivax* with or without the addition of cytokines. The results of these experiments are detailed in results.

II. Results

A. Adaptation of a Mefloquine resistant *P. falciparum* C2A clone to *Aotus* monkeys.

Mefloquine resistant strains of *P. falciparum* have been detected along the Cambodia-Thailand border in Asia. These strains have been studied *in vitro* but until now adaptation to Aotus has been unsuccessful. The purpose of this experiment was to adapt Mefloquine clones to Aotus monkeys in order to do future drug resistant studies *in vivo*. On December 14, 1998 three splenectomized Aotus were inoculated Intravenously (IV) and IP with 1 and 3 mls respectively of cultured *P. falciparum* parasites strains WR75 and clones C2A and C2B brought from WRAIR. Seventy three days Post-Inoculation (PI) the C2A inoculated monkey (89005) became positive with a peak parasitemia of 10,500 x *ul* on day 84 PI selfcuring on day 106 PI. This animal died of cardiac arrest on day 124 PI. Blood from this animal was further passage six times into splenectomized an intact Aotus as shown in Table 1. An aliquoat of frozen stabilate was sent to WRAIR for further passage *in vitro* and for genetic analysis.

B. Reversal of Chloroquine resistance with the co-administration of Prochlorperazine (WR280001AC; BN 43106) and Chloroquine (WR1544 BM;AR 20613) against infections of the AMRU-1 strain (CQR) of *Plasmodium vivax*.

Previous studies with a CQR *P. falciparum* have shown that it is possible to achieve *in vivo* reversal of CQR by the co-administration of Prochlorperazine and Chloroquine, as evidenced by infection cure. Neither drug alone effects such cure. In one study with the CQR AMRU-1 strain of *P. vivax*, data indicated that Prochlorperazine administered at 20 mg/kg x 3 days in combination with Chloroquine at 10.0 mg/kg x 3 days cured 2 of 3 infections, whereas, Chloroquine alone at 10 mg/kg did not. This study was designed to repeat and reconfirm if CQR of the AMRU-1 strain can be reversed *in vivo* by Prochlorperazine plus Chloroquine.
On June 6, 1999 each of 10 *Aotus l. lemurinus*, cured of *P. falciparum,* males and females, were divided in four groups of three animals each and inoculated intravenously with 5×10^6 of *P. vivax* AMRU-1 strain parasites. When parasitemias approximated 5,000 per cmm, oral treatment was initiated for five days with the drugs alone or in combination as shown in Table 2. This time results demonstrated that 3/3 monkeys from group 3 cleared and cured parasitemias on the second day after treatment and remained negative for more than 44 days PI. This experiment re-confirmed reversal of chloroquine resistance of *P. vivax* AMRU-1 using the combination of Phroclorperazine plus Chloroquine.

C. Passage of the AMRU-1 strain (CQR) and the SAL-1 strains of *P. vivax* in Aotus for *in vitro* drug susceptibility testing and efficacy of Artelinic acid *in vivo*.

The emergence of Chloroquine resistant *P. vivax* is a newly emerging problem of antimalarial drug resistance. Since the first description of resistant *P. vivax* in Papua New Guinea, other resistant isolates have been confirmed in Oceania, in Southeast Asia, and South America. Due to the difficulty of growing *P. vivax in vitro*, previous studies of drug resistance in *P. vivax* have been limited to clinical studies or with the one chloroquine resistant isolate that has been adapted to grow in Aotus monkeys. Therefore little work has been done to understand the underlying mechanism of resistance to chloroquine in *P. vivax.*

The purpose of this experiment was to expand upon the *in vivo* data obtained in previous experiment by taking *P. vivax* isolates from the monkeys and conducting resistance reversal studies *in vitro.*

On 24 June 1999, two Aotus cured of *P. falciparum* malaria infection were inoculated, intravenously with one ml of infected blood of the AMRU-1 and Sal-1 strains of *P. vivax*: Parasitemia were followed by daily blood smears and 1.5 ml of blood was collected aspetically once the peak parasitemia was reached for the *in vitro* studies.

Treatment was initiated on day 12 PI with 2 mg/kg of Artelinic Acid for three days. As shown in Tables 4-5 the AMRU-1 inoculated Aotus did not respond to treatment and remained positive up to 32 PI (18 days Post-Treatment). In contrast, the Sal-1 inoculated Aotus cleared parasitemiass six days after finishing treatment and remained negative for more than 17 days. Results of the *in vitro* studies are pending.

D. Oral administration of Artelinic acid (WR 255663AK) against infections of *P. falciparum* FVO in Aotus monkeys.

The artemisinin antimalarial drugs generally are considered the most important class of drugs for the future control of infections due to multiple drug resistant *P. falciparum*. These drugs, originally isolated by Chinese scientists from sweet wormwood (*Artemisia annua*), have been used for the past decade in Asia and some other malaria endemic areas without the benefit of registration by drug regulatory authorities in the US or Europe. Artemisinin derivatives such as Artesunate, Artemether, and Dihydroartemisinin have been used primarily on the basis of limited preclinical data that is available on the class from the Chinese.

Although many of the preclinical efficacy studies have been completed for Artelinic acid, several important projects remain to be completed in Aotus monkeys infected with human malaria isolates. In this study we conducted a dose ranging study of Artelinic acid for the oral treatment of *P. falciparum* infections.

On July 7, 1999, each of ten malaria naïve Aotus were inoculated with 50×10^3 *P. falciparum* FVO malaria parasites IV and divided in five groups of two monkeys each.

As shown in tables 6 and 7, a supression on parasitemia was observed in 1/2 Aotus from group 1 on the second day of treatment. However, one animal died on day 5 after treatment and no effect was observed in the other one until it had to be treated at the next dose level of 8 mg/kg for three days on day 14 PI. In the other groups parasitemia was cleared between days 1-4 after treatment. However all animals recrudesce between days 3-8 after treatment.

E. Efficacy of oral and intravenous administration of falcipain (APC3317) against infections of *P. falciparum* FVO in Aotus monkeys..

The cysteine protease falcipain is required for the degradation of hemoglobin by malaria parasites. Inhibitors of falcipain block hemoglobin degradation and development by erythrocytic parasites. The vinyl sulfone APC-3317 inhibits falcipain at low nanomolar concentrations. The compound also blocked the hydrolysis of hemoglobin and development of *P. falciparum* parasites in vitro and cured 40% of *Plasmodium vinckei*-infected mice. Primate studies are desired to test, for the first time, the efficacy of falcipain inhibitors against *P. falciparum* in vivo.

On February 4, 2000, each of 5 malaria-naïve *Aotus*, males and females, weighing from (811-1003) grms, were inoculated intravenously with 50 x 10^3 FVO *P. falciparum* and divided into two groups of two monkeys each and one control. As shown in Table 8 no effect of the drugs at 50 mg/kg by either of the two routes was observed over the parasitemia course. One animal from the intravenous group died during the injection on the first day of treatment due to toxic effects. The other one from this group died on the second day post treatment (PT). In the oral group one animal died on the third day PT. Neurological signs and cardiorespiratory arrest were observed before death in the IV group treated animals.

F. Oral administration of Artelinic acid (WR 255663AK) vs Artesunic Acid (BM 17174) against infections of *P. falciparum* FVO in Aotus monkeys.

On November 7, 1999 each of twenty four (24) malaria naïve Aotus were divided in two groups of twelve animals each and inoculated with 50 x 10^3 *P. falciparum* FVO malaria parasites IV and further divided into four groups of three monkeys each and treated with Artelinic Acid or Artesunic Acid as shown in Table 10.

Results of the experiment are summarized in Tables 10-13. Briefly, in the Artelinic Acid treated animals, all cleared parasitemias between days –1-1 after treatment. Recrudescence occurred in all between days 5-12 after finishing treatment and was dose dependent. Two animals from the Artelinic Acid treated group that received 32 mg/kg and one of them that was re-treated at 64 mg/kg died with signs of renal failure on days 26 and 21 PT respectively. Organs including kidneys will be send to WRAIR for pathology. On the Artesunic Acid treated animals, all cleared their parasitemias between –1-1 days after treatment. However, recrudescence occurred in all, between 6-14 days after treatment except for one animal of the 32 mg/kg group which remained negative for 116 days, when the experiment finished.

G. Priming for *P. vivax* Antigens by Prior Infection with *P. falciparum* in *Aous* monkeys.

Aotus monkeys previously infected with *P. falciparum* (and cured) had greater immune responses to primary immunization with *P. vivax* antigens than is usually seen. This raises concerns that *P. vivax* antigens might not be best tested in monkeys that have a history of *P. falciparum* infection. The objective of this experiment was to determine whether prior exposure to blood stage infection with *P. falciparum* increases the immune response to subsequent primary immunization with *P. vivax* antigens.

On May 5, 1999 each of eight Aotus, four naïve and four previously exposed *to P falciparum* were infected with 10,000 parasites of the Sal-1 strain of *P. vivax* and divided in two groups of four monkeys each. As shown in Table 14, all animals were parasitemic between days 4 and 6 PI. Peak parasitemias were reached in Group 1 between days 13-14 with a minimum of 4.51×10^3 parasites x *ul* and a maximum of 78.53×10^3 parasites x *ul*. In Group 2 peak parasitemias were reached between days 14 and 18 with a minimum of 21.14×10^3 parasite x *ul* and a maximum of 72.48×10^3 parasites x *ul*. Only one animal from Group 1 had to be treated due to a low Hto reading. Parasitemias cleared in Group 1 (Previously exposed to *P. falciparum*) animals between days 27-37 PI. in contrast Group 2 animals (Naïve for malaria) cleared parasitemias between days 26-34 but two animals recrudesce on day 36 PI clearing between days 40-44 PI. No recrudescence was observed in group 1 animals after 64 days PI. Serological tests are pending in this experiment.

H. Passive transfer of anti-EBA-175 Region II protein monoclonal antibodies to *Aotus* monkeys infected with *Plasmodium falciparum.*

On 12 March, 1999, four monkeys were inoculated with 10,000 parasites of an FVO strain of *P. falciparum* in order to test if a Mouse monoclonal antibody directed against region II of EBA-175 from *P. falciparum* was able to provide protection to *Aotus* monkeys when infused IV during the early stages of a *P. falciparum* blood-stage infection. The experiment consisted of two groups of 4 monkeys each that on the last day of pre-patency received by an IV bolus, 4 mls of 15 mg/ml mouse monoclonal antibody in PBS. The same dose was administered again 24, 48 and 72 hours later for a total dose of 240 mg. The controls which consisted of 4 monkeys received by IV bolus 4 mls of 15 mg/ml of control mouse monoclonal antibody in PBS. The same dose was administered again 24, 48 and 72 hours later for a total dose of 240 mg. Results of this experiment are summarized in Table 15. Briefly, In group 1, 3/4 monkeys

were treated with 40 mg/kg of Mefloquine once between days 13-15 PI either for high parasitemias > 400,000 parasites x *ul* or low htos, and only 1 animal with a peak parasitemia of 57,380 parasites x *ul* selfcured on day 20 PI. In contrast, all group 2 animals were treated between days 14 and 17 PI due to parasitemias > 400,000 parasites x *ul.*

I. Immunization of Aotus monkeys against *P. falciparum* malaria with a plasmid encoding region II of EBA-175 followed with by a EBA-175 recombinant protein boost.

This experiment was started on 18 March, 1999 in order to determine if three immunizations with a plasmid encoding region II of EBA-175 followed by one immunization with EBA-175 region II recombinant protein produces protection from blood stage *P. falciparum* infection. The experiment consited of two groups of 6 monkeys and a third group of 3 monkeys. In group 1, all monkeys received three doses of a plasmid encoding EBA-175 (region II), and 500 *ug* of VR1721, a plasmid encoding *Aotus* GM-CSF, solubilized in PBS and delivered ID. Following the three doses of DNA vaccine, the animals received a boosting immunization consisting of baculovirus produced recombinant EBA-175 Region II protein emulsified in Montanide 720 containing 500 *ug* of CpG oligodeoxynucleotide 1968. The animals received half of protein dose SC along the flanks, and half IM in the quadruceps. Group 2 received three doses of one ml containing 500 *ug* of VR1050 the backbone plasmid of VR2527, and 500 *µg* of VR1721, a plasmid encoding *Aotus* GM-CSF, solubilized in PBS and delivered ID. These animals were then boosted with Montanide 720 and CpG (Adjuvant control), delivered both SC and IM as above. Group 3 was treated the same as Group 1 except that it received 100 *ug* of protein delivered IM only. Challenge with 10,000 parasites of a *P. falciparum* FVO strain was carried out on October 12, 1999. Results of this experiment are shown in Table 16. Briefly, on day five PI all monkeys became positive. The naïve control became positive on day 4 PI. Treatment with 20 mg/kg of mefloquine was initiated on day 11 PI in 3/6 monkeys from group 2 and 1/5 from group 1. Two out of three monkeys from group 3 were treated on this day also. By day 12 PI another monkey from group 2 and two monkeys from group 1 were treated. At that time the naïve control was also treated. The last monkey from group 2 was treated on day 15 PI. The remaining two monkeys from group 1 were treated on days 17 and 18 PI respectively, due to low htos readings. However, one of these monkeys died three days after treatment. Only 1 monkey from group 3 selfcured on day 25 PI and remained negative for the rest of the experiment. Serological results are pending.

J. Immune induction against Malaria infection in Aotus monkeys by topical ocular administration of a plasmid DNA vaccine encoding an AMA-1 *P. falciparum* blood stage antigen.

The ocular surface represents a unique milieu that is constantly exposed to toxic, antigenic and microbiological insults. In humans, the conjuctiva has been linked to an opened-up lymph node, with the exception that the antigens or infectious agents must transmigrate across the conjuntival epithelium before encountering the vast majority of immunocompetent cells within the substantia propia. Recently Plasmid DNA vaccines have been administered by the ocular route in mice, providing protection against a challenge with Herpes simplex virus. This hypothesized that Immunization of Aotus monkeys with a plasmid DNA vaccine directed against blood stage *P. falciparum* determinants by the ocular route will protect monkeys against a blood stage challenge. For this purpose on 18 March 1999, two naïve Aotus monkeys were immunized by the ocular route in both eyes with 50 *ul* of a dilution containing an AMA-1 plasmid vaccine three times at one month intervals. The animals were bled every two weeks and each time immediately before immunization. No seroconversion was observed in this experiment.

K. Effect of formulation in 150 mM Na phosphate buffer versus phosphate buffered saline on immunogenicity of DNA vaccines in Aotus monkeys.

Vival Inc has reported *in vivo* expression and improved immunogenicity of DNA vaccines formulated in Na phosphate as opposed to the standard formulation in phosphate buffered saline. The aim of this study was to confirm improved immunogenicity in primates in order to decide whether to formulate DNA vaccines in Na phosphate for planned human trials.

Each of 16 *P. falciparum* and *vivax* cured Aotus monkeys were divided in two groups of 8 monkeys each and immunized as follows: Group 1 received 500 ug/dose x 3 doses of VR2516 in PBS delivered ID to the lower back in six different sites. Group 2, received 500 ug/dose x 3 doses of VR2516 in 150 mM Na phosphate delivered ID to the lower back in six different sites. All animal received three doses of the plasmids at one month intervals. No challenge was carried out in this experiment. Results of this experiment are pending.

CONCLUSIONS

A C2A clone of a Mefloquine resistant *Plasmodium falciparum* strain was adapted to splenectomized and intact *Aotus*.

Chloroquine resistance reversal was achieved in 3/3 *Aotus* infected with the AMRU-1 strain of *Plasmodium vivax* by using chloroquine at 10mg/kg and prochlorperazine at 20 mg/kg in combination.

Artelinic Acid (WR255663AK;BM04131) when given orally at 2 mg/kg x three days suppressed infections of the AMRU-1 (CQR) but cleared SAL-1 strains of *P. vivax* in Aotus monkeys.

Artelinic Acid (WR255663AK;BM04131) administered orally at 2-24 mg/kg x three days was effective against infections of *P. falciparum* FVO strain in Aotus monkeys.

Orally or intravenously administered falcipain (APC3317) was ineffective against infections of *P. falciparum FVO*.

Artelinic Acid and Artesunate were effective against infections with *P. falciparum* FVO in Aotus monkeys.

Passive transfer of anti-EBA-175 Region II protein monoclonal antibodies was not effective at controlling parasitemia in *Aotus* monkeys infected with *P. falciparum.*

Immunization with a plasmid encoding region II of EBA-175 followed with by a EBA-175 recombinant protein boost partially protected Aotus monkeys against *P. falciparum* malaria.

Topical ocular administration of a plasmid DNA vaccine encoding an AMA-1 *P. falciparum* blood stage antigen did not induce an immune response in Aotus monkeys.

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TABLE 1

Adaptation of *Plasmodium falciparum* C2A clone in Aotus monkeys

PASSAGE LEVEL	MONKEY	DONOR MONKEY	DATE INOC	PARASITEMIA X 10^3 PREPATENT PERIOD	PEAK	DAY PI	TREATMENT DRUG	REGIMEN	DAY PI	RESULT OF TREATMENT	RECRUDESCE DAY/PI	RETREATMENT DRUG	RETREATMENT REGIMEN	RETREATMENT RESULT	FOLLOW UP DAYS/PI	DISPOSITION (day)
0	89005*	Culture	12/14/98	72	10.5	84	none	none	none	none	none	none	none	none	124	died(124)
1	92015*	89005	2/26/99	1	20.1	22	WR142490	40m/g/kg/3/days	73	cleared and cured	none	none	none	none	187	cured(93)
2	88011*	92015	3/15/99	0	12	6	none	none	none	none	none	none	none	none	15	died(15)
2	92034*	92015	3/15/99	0	85.1	11	WR142490	40 mg/kg once	11	suppresed	(44)(95)	none	none	none	221	self-cured(114)
2	12971	92015	3/15/99	0	0.02	4	none	none	none	none	none	none	none	none	115	self-cured(15)
3	12987	92034	6/8/99	3	0.01	4	none	none	none	none	none	none	none	none	124	self-cured(4)
3	93014*	92034	8/12/99	2	121.5	8	WR255663	8.0 mg/kg/3/days	8	suppresed	16 36	WR255663 WR00297308	16MG/KG/3/days 20MG/KG/3/days	suppressed none	44	died (45)
3	12955	92034	8/12/99	2	1.7	11	none	none	none	none	none	none	none	none	124	self-cured(19)
4	12956	12955	8/25/99	2	0.38	7	none	none	none	none	none	none	none	none	111	self-cured(14)
5	12961	12956	9/4/99	11	0.96	28	none	none	none	none	none	none	none	none	101	self-cured(35)

*=Splenectomized

TABLE 2

DETAILED ACTIVITY OF PROCHLORPERAZINE* (WR280001AC;BN43106) AND CHLOROQUINE** (WR1544BM;AR20613) AGAINST INFECTIONS OF THE AMRU-1 STRAIN (CQR) OF *Plasmodium vivax* in Aotus Monkeys

	RX INITIATED			PARASITEMIA PER CMM X 10^3										
MONKEY #	DAY P.I.	DAY PAT.	MG/KG	DAY PRE .	DAY OF RX					DAY POST RX				
				RX	1	2	3	4	5	1	2	3	4	Days Neg.
12865	9	4	10**	1.8	7.5	11.4	45.3	31.71	54.3	27.1	31.7	44.3	36.2	none
12866	9	4	10**	0.86	4	2.59	2.06	0.89	0.01	0.01	0	0	0	3
12904	9	2	10**	0.76	2.2	8.94	13.01	10.57	6.08	8.8	8	3.53	5.01	none
12882	9	3	20*	0.62	1.89	5.02	12.96	12.08	24.9	25.4	11.7	2.8	0.56	none
12870	9	4	20*	0.58	2.91	5.12	25.67	19.63	29.6	22.65	8.1	6.04	1.34	none
12876	9	3	20*	1.36	6.04	11.7	24.1	27.18	64.9	19.91	8.8	8.81	1.26	none
12875	9	2	20*	0.71	5.12	5.1	6	0.21	0.01	0.01	0	0	0	116
12903	9	4	20* 10**	1.3	2.01	5.96	19.12	0.52	0.01	0.01	0	0	0	116
12880	9	2	20* 10**	0.28	2.86	2.6	13.5	1.32	0.01	0.01	0	0	0	81***
12869		4	control 10**	0.74	3.02	3.91	10.57	22.5	25.6	25.6	15.1	7.55	4.5	none

*Phrochlorperazine 20 mg/kg
**Chloroquine 10 mg/kg
***=Out of experiment

TABLE 3

SUMMARY OF ACTIVITY OF PROCHLORPERAZINE* (WR280001AC;BN43106) AND CHLOROQUINE** (WR1544BM;AR20613) AGAINST INFECTIONS OF THE AMRU-1 STRAIN (CQR) OF *Plasmodium vivax* in Aotus Monkeys

MONKEY No.	Daily Dose x 5 mg/kg	Response of Parasitemia to Rx			Days from initital Rx to parasite Clearance	Days from final Rx to Recrudescence	Notes No. of Days Neg.
		None	Suppresed	Cleared			
12865	10**	X					
12866	10**	X					
12904	10**			X	6	4	3
12882	20*	X					
12870	20*	X					
12876	20*	X					
12875	20* 10**			X	6		116
12903	20* 10**			X	6		116
12880	20*			X	6		81***
12869	control						

*Phrochlorperazine 20 mg/kg
**Chloroquine 10 mg/kg
***=Out of experiment

TABLE 4

DETAILED ACTIVITY OF ARTELINIC ACID (WR255663AK;BM04131)
AGAINST INFECTIONS OF THE AMRU-1 (CQR)* AND SAL-1** STRAINS OF *Plasmodium vivax* in Aotus monkeys.

	RX INITIATED			PARASITEMIA PER CMM X 10^3													Days
				DAY PRE .	DAY OF RX			DAY POST RX									
MONKEY	DAY P.I.	DAY PAT.	MG/KG	RX	1	2	3	1	2	3	4	4	6	7	8	9	Neg.
12915*	12	12	2	32.71	39.4	26.6	10.5	6.63	1.99	1.59	2.06	2.7	0.88	1.86	1.42	0.82	87
12926**	12	12	2	24.66	19.12	19.71	5.19	2.76	0.98	0.44	0.01	0.01	0.01	0	0	0	94

TABLE 5

SUMMARY OF ACTIVITY OF ARTELINIC ACID (WR255663AK;BM04131)
AGAINST INFECTIONS OF THE AMRU-1* (CQR) AND SAL-1** STRAINS OF *Plasmodium vivax* in Aotus monkeys.

MONKEY #	Daily Dose x 3 days Mg/Kg	Response of parasitemia to Rx			Days from final Rx to parasite clearance	Days from final Rx to recrudescence	Notes No. of days negative
		None	Suppressed	Cleared			
12915	2			X	18		87
12926	2			X	6		94

TABLE 6

DETAILED ACTIVITY OF ARTELINIC ACID (WR255663AK;BM04131)
AGAINST INFECTIONS OF *Plasmodium falciparum* FVO strain in Aotus monkeys.

	RX INITIATED			PARASITEMIA PER CMM X 10^3 DAY PRE	DAY OF RX			DAY POST RX								Days
MONKEY	DAY P.I.	DAY PAT.	MG/KG	RX	1	2	3	1	2	3	4	5	6	7	8	9	Neg.
12982	7	4	2	1.2	51	5	0.01	0.01	0.01	0.01	0.01	0.01	DIED				0
12986	7	4	2	0.38	39.26	2.89	8.06	3.89	36.29	72.68	98.15	320.5***	191.7***	130.5***	4.99	2.66	0
12980	7	4	8	0.82	36.2	4.5	0.01	0	0	0	0	0	0	0	0.01	0.01	7
12981	7	4	8	0.34	24.16	2.99	0.01	0	0	0.01	0.01	0.01	0.02	12.08***	80***	2.8***	2
12988	7	4	16	0.36	43.7	17.8	2.1	1.01	0.01	0.01	0	0	0	0	0.01	0.02	4
12991	7	4	16	0.33	42.2	12.96	1.05	0.01	0.01	0.01	0	0	0	0	0.01	0.01	4
12985	7	4	24	0.89	34.7	10.5	0.01	0	0	0	0	0	0	0	0.01	0.01	7
12979	7	4	24	0.79	27.18	13.09	4.11	0.01	0.01	0.01	0	0	0	0	0.01	0.48	4
12990	11	8	4	0.29	200.17	96.64	49.83	19.6	40.1***	147***	6.04***	2.69	0.01	0	0	0	0
12989	10	7	4	0.39	289.76	87.58	39.26	63.42	85.5	86	320***	161***	40.5***	12.88	0.01	0.01	0

***=Retreatment at next dose level

TABLE 7

SUMMARY OF ACTIVITY OF ARTELINIC ACID (WR255663AK;BM04131)
AGAINST INFECTIONS OF *Plasmodium falciparum* FVO strain in Aotus monkeys.

MONKEY #	Daily Dose x 3 days Mg/Kg	Response of parasitemia to Rx			Days from final Rx to parasite clearance	Days from final Rx to recrudescence	Notes No. of days negative
		None	Suppressed	Cleared			
12982	2		X				DIED
12986	2	X					
12980	8			X	1	8	7
12981	8			X	1	3	2
12988	16			X	4	5	4
12991	16			X	4	5	4
12985	24			X	1	8	7
12979	24			X	4	5	4
12990	4	X					
12989	4	X					

TABLE 8

DETAILED ACTIVITY OF ORALLY vs INTRAVENOUSLY ADMINISTERED FALCIPAIN (APC3317) AGAINST INFECTIONS OF *Plasmodium falciparum* FVO STRAIN IN AOTUS MONKEYS

			RX INITIATED		DAY PRE	PARASITEMIA PER cmm X 10^3 DAY OF RX										Days
						1		2		3		DAY POST RX				
MONKEY #	ROUTE	DAY P.I.	DAY PAT.	MG/KG	RX	am	pm	am	pm	am	pm	1	2	3	4	Neg.
13001	Oral	8	8	50	8	6.1	33.2	129	83	138.9	343.9	273.6	579.8*		DIED	0
13000	Oral	8	8	50	6.8	9	39.2	143.4	18.1	73.9	214.4	143.6	675.5*			0
13002	IV	8	8	50	4.1	13.5	2.4 DIED									0
12972	IV	8	5	50	4	16.6	87	96.6	45.3	39.2	134.3	114.7	DIED			0
13004	None			None	2.8	4.5	31.7	63.4	16.9	113.2	374.4	168.9	525.3*			0

*=Treated with Mefloquine 20 mg/kg

TABLE 9

SUMMARY OF ACTIVITY OF ORALLY VS INTRAVENOUSLY ADMINISTERED FALCIPAIN (APC3317) AGAINST INFECTIONS OF *Plasmodium falciparum* FVO strain in Aotus monkeys.

MONKEY #	Route	Daily Dose x 3 days Mg/Kg	Response of parasitemia to Rx			Days from final Rx to parasite clearance	Days from final Rx to recrudescence	Notes No. of days negative
			None	Suppressed	Cleared			
13001	Oral	50	X					DIED
13000	Oral	50	X					
13002	IV	50	X					DIED
12972	IV	50	X					DIED
13004	None	50						

TABLE 10

DETAILED ACTIVITY OF ARTELINIC ACID* (WR 255663AK; BM04131) VS ARTESUNIC ACID (BM 17174) AGAINST INFECTIONS OF *Plasmodium falciparum* FVO IN AOTUS MONKEYS

MONKEY	RX INITIATED			PARASITEMIA PER cmm X 10^3									Days
	DAY P.I.	DAY PAT.	mg/kg	DAY PRE	DAY OF RX			DAY POST RX					
				RX	1	2	3	1	2	3	4	5	Neg.
92031	8	3	32*	3.81	2.91	0.01	0	0	0	0	0	0	10
95007	8	4	32*	0.72	0.77	0.01	0	0	0	0	0	0	10
93020	8	3	32*	0.66	2.81	0.01	0	0	0	0	0	0	12
12994	8	4	24*	10.5	16.6	0.51	0.01	0	0	0	0	0	9
93031	8	4	24*	2.1	1.35	0.01	0	0	0	0	0	0	10
91009	8	4	24*	1.04	3.11	0.91	0.01	0	0	0	0	0	8
95001	8	4	16*	0.81	1.89	1.75	0.01	0	0	0	0	0	9
12996	8	4	16*	9	13.5	1.18	0.01	0	0	0	0	0	7
93017	8	4	16*	2.02	12	0.31	0.01	0	0	0	0	0	8
89061	8	4	8*	0.87	9.01	0.24	0.01	0	0	0	0	0	5
93034	8	4	8*	0.82	0.86	0.01	0	0	0	0	0	0.01	5
93033	8	2	8*	0.01	0.02	0.36	0.01	0	0	0	0	0.01	4
91020	CONTROL			0.51	0.67	45.12	31.5	192.7	504**	243**	65**	9.5	7
92019	CONTROL			0.98	1.09	33	9.18	150.9	410**	199**	63**	7.5	4
93030	CONTROL			0	0	0.01	0.01	0.01	9.04	19.6	116.2	24.2	14

**=Treatment Artelinic Acid 32 mg/kg

TABLE 11

DETAILED ACTIVITY OF ARTELINIC ACID (WR 255663AK; BM04131) VS ARTESUNIC ACID** (BM 17174) AGAINST INFECTIONS OF *Plasmodium falciparum* FVO IN AOTUS MONKEYS

					PARASITEMIA PER cmm X 10^3								
		RX INITIATED		DAY PRE	DAY OF RX			DAY POST RX					Days
MONKEY	DAY P.I.	DAY PAT.	mg/kg	RX	1	2	3	1	2	3	4	5	Neg.
12995	8	4	32**	2.8	19.5	0.24	0.01	0	0	0	0	0	13
95020	8	3	32**	0.59	1.52	0.01	0	0	0	0	0	0	116
93026	8	3	32**	1.77	1.96	0.01	0	0	0	0	0	0	12
92004	8	4	24**	1.1	1.35	0.01	0	0	0	0	0	0	10
90034	8	4	24**	2.01	5.99	0.01	0	0	0	0	0	0	11
96025	8	4	24**	0.32	1.59	0.01	0	0	0	0	0	0	12
94014	8	4	16**	1.15	1.01	0.01	0	0	0	0	0	0	6
95011	8	4	16**	1.55	1.14	0.24	0.01	0	0	0	0	0	8
96021	8	4	16**	0.28	1.09	0.01	0	0	0	0	0	0	9
97003	8	4	8**	2.09	6.01	1.15	0.01	0	0	0	0	0	5
94011	8	4	8**	1.99	0.99	0.41	0.01	0	0	0	0	0	5
94006	8	4	8**	5.19	30.2	10.5	0.01	0	0	0	0	0	5

TABLE 12

SUMMARY OF ACTIVITY OF ARTELINIC ACID* (WR255663AK;BM04131) VS ARTESUNIC ACID (BM 17174) AGAINST INFECTIONS OF *Plasmodium falciparum* FVO strain in Aotus monkeys.

MONKEY #	Daily Dose x 3 days Mg/Kg	Response of parasitemia to Rx None	Suppressed	Cleared	Days from final Rx to parasite clearance	Days from final Rx to recrudescence	Notes No. of days negative
92031	32*			X	-1	10	10
95007	32*			X	-1	10	10
93020	32*			X	-1	12	12(Died day 31 PI)
12994	24*			X	1	10	9
93031	24*			X	-1	10	10
91009	24*			X	1	9	8
95001	16*			X	1	10	9
12996	16*			X	1	8	7
93017	16*			X	1	9	8
89061	8*			X	1	6	5
93034	8*			X	-1	5	5
93033	8*			X	1	5	4
91020	32*			X	3	10	7
92019	32*			X	3	7	4
93030	32*			X	4	None	14 (Died day 35 PI)

Retreatment was carried out at next highest dose

TABLE 13

SUMMARY OF ACTIVITY OF ARTELINIC ACID (WR255663AK;BM04131) VS ARTESUNIC ACID** (BM 17174) AGAINST INFECTIONS OF *Plasmodium falciparum* FVO strain in Aotus monkeys.

MONKEY #	Daily Dose x 3 days Mg/Kg	Response of parasitemia to Rx None	Suppressed	Cleared	Days from final Rx to parasite clearance	Days from final Rx to recrudescence	Notes No. of days negative
12995	32**			X	1	14	13
95020	32**			X	-1	None	116
93026	32**			X	-1	12	12
92004	24**			X	-1	10	10
90034	24**			X	-1	11	11
96025	24**			X	-1	12	12
94014	16**			X	-1	6	6
95011	16**			X	1	9	8
96021	16**			X	-1	9	9
97003	8**			X	1	6	5
94011	8**			X	1	6	5
94006	8**			X	1	6	5

Retreatment was carried out at next highest dose

TABLE 14

DETAILED PARASITEMIA OF AOTUS INFECTED WITH *Plasmodium vivax* SAL-1 STRAIN
TO DETERMINE IF PRIOR EXPOSURE TO *Plasmodium falciparum* PRIME AOTUS TO *P. vivax* ANTIGENS

Parasitemia x ccmm x 10^3

MONKEY	GROUP	DAY/PI	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
12920	1		0	0	0	0	0	0	0.01	0.01	0.01	0.01	0.74	1.5	3.94	7.5	18.1	11.52
12921	1		0	0	0	0	0.01	0.01	0.01	0.01	0.01	0.01	0.22	1.22	1.9	4.51	2.04	1.09
12922	1		0	0	0	0	0.01	0.01	0.01	0.01	1.01	1.81	6.51	15.1	29.75	57.38	78.52	49.35
12923	1		0	0	0	0	0	0.01	0.01	0.02	0.01	0.01	0.98	3.98	7.85	30.2	48.32	19.63
12973	2		0	0	0	0	0	0.01	0.01	0.01	0.02	0.8	2.54	3.9	11.52	34.73	72.48	40.77
12974	2		0	0	0	0	0.01	0.01	0.01	0.01	0.02	0.62	0.78	4.35	9.75	17.09	21.14	16.61
12977	2		0	0	0	0	0	0	0.01	0.01	0.02	0.64	1.78	2.54	8.82	22.14	23.09	24.16
12978	2		0	0	0	0	0	0	0.01	0.01	0.02	0.01	0.39	1.01	2.4	12.08	20.96	15.1

MONKEY	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
12920	16.72	19.5	12.98	9.06	3.05	2.42	0.68	0.01	0.01	0.01	0.01	0	0	0	0	0	0	0
12921	1.05	0.7	0.36	0.01**	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0
12922	27.18	32.25	27.18	6.01	1.89	1.12	2.18	0.01	0.01	0.02	0.98	0.59	0.49	0.29	0.62	0.36	0.01	0.01
12923	30.2	23.25	15.82	1.75	0.71	0.01	0.01	0.01	0.01	0.01	0	0	0	0	0	0	0	0
12973	36.24	39.26	21.14	6.89	12.08	10.02	6.4	2.11	0.94	0.86	0.01	0.01	0	0	0	0	0.01	0
12974	7.11	4.34	2.55	0.81	0.95	0.89	0.69	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0
12977	10.57	6.3	1.99	0.59	1.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
12978	33.22	19.63	40.77	15.1	13.66	15.1	4.08	1.21	4.9	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0	0

MONKEY	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	(Days) (Neg) Disp.
12920	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	109
12921	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Treated**
12922	0.01	0.01	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
12923	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	111
12973	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	104
12974	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	104
12977	0	0	0.01	0.01	0.01	0.1	0.01	0.01	0.01	0.01	0.01	0	0	0	0	0	0	92
12978	0	0	0.01	0.01	0.01	0.01	0	0	0	0	0	0	0	0	0	0	0	(43)Died

**= Treated with mefloquine 20 mg/kg

TABLE 15

DETAILED PARASITEMIA OF PASSIVE TRANSFER OF ANTI-EBA-175 REGION II PROTEIN MONOCLONAL ANTIBODIES TO AOTUS MONKEYS INFECTED WITH *Plasmodium falciparum* FVO

MONKEY	GROUP	Parasites x ccmm x 10^3														
DAY/PI		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
11969	1	0	0	0	0	0	0	0.01	0.01	13.5	19.6	15.1	27.1	30.2	57.3	90.6
12868	1	0	0	0	0	0	0	0	0.01	1.1	21.1	40.7	80	52.9	211.4*	
12867	1	0	0	0	0	0	0	0	0.01	19.3	96.6	247.6	549.6*			
12918	1	0	0	0	0	0	0.01	0.01	1	27.1	51.3	145.9	334.8	676.4*		
12930	2	0	0	0	0	0	0.01	0.01	1.6	57.3	138.9	131.3	281.8	300.9*		
12936	2	0	0	0	0	0	0.01	0.01	1.3	61.9	187	248	366	626.4*		
12917	2	0	0	0	0	0	0.01	0.01	0.01	6	6	24.1	66.4	92.1	229.5	283.1
12065	2	0	0	0	0	0	0	0.01	0.01	25.6	70.9	154	265.2	321.8*		

DAY/PI	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Days Neg. Disp.
11969	49.8	2	0.97	0.01	0.01	0	0	0	0	0	0	0	0	0	0	48(Died)
12868																
12867																
12918																
12930																
12936																
12917	187.2*															
12065																

*=Treatment with mefloquine 20 mg/kg

TABLE 16

DETAILED PARASITEMIA OF AOTUS IMMUNIZED WITH A PLASMID ENCODING REGION II OF EBA-175 FOLLOWED BY A EBA-175 RECOMBINANT PROTEIN BOOST AND INFECTED WITH *Plasmodium falciparum* FVO

		DAY PI	Parasites x ccmm x 10^3													
MONKEY	GROUP	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
12944	3	0	0.01	0.01	0.02	0.34	8	115.5	400.2*							
12941	3	0	0.01	0.01	0.02	0.68	40.7	112.5	121.1	198.4	122.3	93.6	63000	63040	51340	12080
12942	3	0	0.01	0.01	0.02	1.7	246	372	497.3*							
12834	1	0	0.01	0.01	0.01	0.01	2.2	2.8	9	147	60.7	259.7	200500	100500	85520*	
12945	1	0	0.01	0.01	0.01	0.01	10.5	2.4	18	36	77	61.5	82560	117000	98150	49550*
12946	1	0	0.01	0.01	0.02	0.01	46.8	58.5	132.8	401.9						
12948	1	0	OUT													
12947	1	0	0.01	0.01	0.02	0.01	54.3	60.9	386.5	816.0*						
12951	1	0	0.01	0.01	0.02	0.96	73.9	116.2	410.8							
12952	2	0	0.01	0.01	0.02	0.01	58.5	70.5	152.5	374.2	303.5	299	204110*			
12957	2	0	0.01	0.01	0.02	0.01	7.6	98.1	401*							
12959	2	0	0.01	0.01	0.02	0.19	63	22	416.1*							
12960	2	0	0.01	0.01	0.01	0.66	87	63.4	400.1*							
12966	2	0	0.01	0.01	0.01	0.01	27	60	205.5	501.9*						
12967	2	0	0.01	0.01	0.01	0.01	8	1.6	42	318.1	326.1	748.9*				
12992	CONTRO	0.01	0.01	0.01	0.01	0.01	31.5	55.8	96	400.0*						

MONKEY	DAY PI	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Days Neg.
12944	3															
12941	3	32110	7160	960	0.01	0.01	0.01	0	0	0	0	0	0	0	0	38
12942	3															
12834	1															
12945	1			DIED												
12946	1															
12948	1															
12947	1															
12951	1															
12952	2															
12957	2															
12959	2															
12960	2															
12966	2															
12967	2															
12992	CONTROL															

*=Treatment with mefloquine 20 mg/kg